Effectiveness of commercially-available antibiotic-impregnated implants

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The aim of this study was to determine the effectiveness of antibiotic-impregnated implants in the prevention of bone infection. We used a model of contaminated fracture in goats to evaluate four treatment groups: no treatment, hand-made tobramycin-impregnated polymethylmethacrylate beads, commercially-available tobramycin-impregnated calcium sulphate pellets and commercially-available tobramycin-impregnated polymethylmethacrylate beads. Three weeks after intraosseous inoculation with streptomycin-resistant *Staphylococcus aureus* tissue cultures showed no evidence of infection in any of the antibiotic-treated groups. All of the cultures were positive in the untreated group. These results show that effective local antibiotic delivery can be obtained with both commercially-available products and with hand-made polymethylmethacrylate beads. The calcium sulphate pellets have the advantage of being bioabsorbable, thereby obviating the need for a second procedure to remove them.

We have previously shown, in an animal model, that calcium sulphate impregnated with 10% tobramycin can prevent bony infection.[1,2] In this study, using the same model, we evaluated the effectiveness of a commercially-available calcium sulphate product that has a lower concentration of tobramycin.

The ideal implant for use in open fractures prevents infection, does not require removal, and supports bone growth. Polymethylmethacrylate (PMMA) impregnated with antibiotics has been used in Europe since the 1970s[3] for patients with infected total joint replacements and open fractures.[3,4] It is not resorbable and has to be removed during a second procedure. Calcium sulphate has been used as a filler for bony defects with good results[5,6] and has been shown to be a good carrier for local antibiotic delivery.[7]

Our hypothesis was that tobramycin-impregnated calcium sulphate pellets would prevent infection in our animal model as well as tobramycin-impregnated PMMA beads.

Materials and Methods

The Institutional Animal Care and Use Committee approved and oversaw this study. All procedures were conducted in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care in accordance with US National Institutes of Health guidelines for the care and use of laboratory animals.

Twenty-nine castrated male Spanish goats with a mean weight of 48.1 kg (SD 0.68) were used. All were tested for tuberculosis, brucellosis and Q fever, and were healthy prior to the study.

Study design. The goats were randomised into four treatment groups. The negative control group received no treatment. The positive control group was treated with hand-made tobramycin-impregnated PMMA beads. These were made by combining one packet (40 g) Palacos (Smith & Nephew Richards Orthopaedics, Memphis, Tennessee) and 20 ml monomer cement with 2.4 g tobramycin sulphate powder (Nebcin) (Eli Lilly & Co., Indianapolis, Indiana). Therefore, the concentration of tobramycin is approximately 4% by weight. The experimental calcium sulphate group was treated with commercially-available tobramycin-impregnated calcium sulphate pellets (Osteoset T, Wright Medical Technology Inc., Arlington, Tennessee) which also contain 4% tobramycin by weight. The experimental PMMA group was treated with commercially-available tobramycin-impregnated PMMA beads (Simplex P with Tobramycin, Stryker Orthopaedics, Mahwah, New Jersey) which contain 1 g of tobramycin per 40 g packet. The concentration of tobramycin is approximately 1.7% by weight when this is mixed with the 20 g monomer. The weights of the substances used were obtained prior to implantation and the total tobramycin volume was calculated.

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Bacterial preparation. A bacterial preparation was made using the *Staphylococcus aureus* strain American Type Culture Collection (ATCC) 29213. We modified the strain to be resistant to streptomycin using a serial plating technique. The most prolific colonies were recovered from streptomycin plates and grown overnight in brain-heart infusion broth. This process was repeated twice; after the third pass the mutant was streaked on plates containing various concentrations of streptomycin (50 to 1000 μl/ml) to ensure resistance. An aliquot of 30 μl of 2.35 (SD 0.19 x 10^6) colony-forming units/ml (CFU/ml) of streptomycin-resistant *Staphylococcus aureus* (*Staph aureus-R*) was chosen as a wound contaminant. In our previous work this quantity was shown to be sufficient to cause infection without producing septicaemia in approximately 90% of non-treated animals.[1,2]

Surgical technique. Under adequate anaesthesia and analgesia the right lower limb was prepared with chlorhexidine gluconate and draped in a sterile fashion. No pre-operative antibiotics were administered. A 2.5 cm longitudinal incision was made over the medial aspect of the proximal tibial metaphysis, centred 2 cm distal and 2 cm medial to the tibial tubercle. The periosteum was elevated and a unicortical 12 mm circular defect was created with a coring reamer. A thrombin-soaked gelatin sponge was used to assist with medullary hemostasis. The defect was inoculated with *Staph aureus-R* and packed with the test material. The goats were allowed activity and feeding *ad libitum* for three weeks.

Necropsy and microbiologic analysis. On day 21 the animals were killed. The bony defect was transected at its mid-portion using a Gigli saw and culture swabs were obtained from the proximal and distal intramedullary canals. A surgical curette was used to obtain 0.5 g of marrow and trabecular tissue, and this and the swab samples were sent for standard qualitative and quantitative microbiological analysis. Each *Staph aureus* isolate was tested for streptomycin resistance to determine whether it was the same strain as the initial inoculum.

Outcome measures. The outcome measure used to define the presence of deep infection was the recovery of *Staph aureus-R* from the cultures. The threshold for infection was set at 10^4 CFU/g of marrow.[8] The cultures that had bacteria present at a concentration of less than 10^4 CFU/g were considered to be contaminated.

Statistical analysis. Analysis of variance was performed to ensure that the groups were comparable with respect to body weight and amount of inoculation. A generalised linear model was used to evaluate the bacterial counts, and the significance level was set at 0.05. When global differences were detected, a step-down Bonferroni adjustment was used for error correction to determine the significance of subsequent comparisons.

Results

None of the animals displayed any signs of systemic sepsis. There were no significant differences between groups with respect to body weight or amount of bacterial inoculum. The total weight of tobramycin implanted for each test group was calculated. In the positive control group, the hand-made PMMA cement was a carrier for 0.107 mg (SD 0.007) of tobramycin. The experimental calcium sulphate pellets implanted 0.065 mg (SD 0.0) of tobramycin. In the experimental PMMA group the commercially-available cement implanted 0.041 mg (SD 0.001) of tobramycin.

Table I. Bacterial growth at 21 days

Goat	Treatment group*	Organism	Bacterial count (CFU/g)†
1	Negative control	*Staph aureus-R*	7.14 x 10^7
2	Negative control	*Staph aureus-R*	9.46 x 10^7
3	Negative control	*Staph aureus-R*	2.30 x 10^7
4	Negative control	*Staph aureus-R*	7.77 x 10^7
5	Negative control	*Staph aureus-R*	4.31 x 10^4
6	Negative control	*Staph aureus-R*	8.54 x 10^6
7	Negative control	*Staph aureus-R*	2.16 x 10^5
8	Negative control	*Staph aureus-R*	7.45 x 10^7
9	Positive control	No growth	0
10	Positive control	No growth	0
11	Positive control	No growth	0
12	Positive control	Coagulase-negative *Staphylococcus*	6.23 x 10^3
13	Positive control	No growth	0
14	Positive control	No growth	0
15	Positive control	Coagulase-negative *Staphylococcus*	6.31 x 10^3
16	Experimental $CaSO_4$	No growth	0
17	Experimental $CaSO_4$	No growth	0
18	Experimental $CaSO_4$	No growth	0
19	Experimental $CaSO_4$	No growth	0
20	Experimental $CaSO_4$	No growth	0
21	Experimental $CaSO_4$	No growth	0
22	Experimental $CaSO_4$	*Staph aureus-S*	1.43 x 10^8
23	Experimental PMMA	No growth	0
24	Experimental PMMA	No growth	0
25	Experimental PMMA	No growth	0
26	Experimental PMMA	No growth	0
27	Experimental PMMA	No growth	0
28	Experimental PMMA	No growth	0
29	Experimental PMMA	No growth	0

* Negative control, no treatment; Positive control, hand-made tobramycin-impregnated polymethylmethacrylate (PMMA) beads; Experimental $CaSO_4$, commercially available tobramycin-impregnated calcium sulphate pellets; Experimental PMMA, commercially-available tobramycin-impregnated polymethylmethacrylate beads

† CFU, colony forming units

All the specimens obtained from the untreated group had necrosis and abscess formation at the site of the cortical defect. They also had positive cultures for *Staph aureus-R* (Table I). The mean growth on medullary culture was 4.38 x 10^7 CFU/g (SD 1.40 x 10^7), and all were considered to be infected.

None of the specimens in the treated groups had gross pathological evidence of infection and the intramedullary culture results revealed no growth for all animals, with three exceptions (see Table I). Two specimens in the positive control group had coagulase-negative *Staphylococcus* growth (each < 10^4 CFU/g) and were considered to be contaminated. One specimen in the experimental calcium sulphate group (Osteoset T) had a significant growth of

streptomycin-sensitive *Staph aureus* (*Staph aureus-S*) 1.43 x 10^8 CFU/g). However, the organism was not the experimentally-induced strain, as it was sensitive to streptomycin. All of the groups treated with antibiotics had significantly fewer bacteria recovered from the intramedullary cultures than the negative control group ($p < 0.0001$).

Discussion

We performed this study to evaluate the *in vivo* performance of commercially-available antibiotic-impregnated calcium sulphate pellets in a contaminated fracture model in goats. We used hand-made antibiotic-impregnated PMMA cement beads as our positive control group, and also added a treatment group treated with commercially-available antibiotic-impregnated PMMA beads. All three of these treatment groups performed well, with no evidence of infection. The untreated group had both gross pathological and confirmed infection with our *Staph aureus-R* bacteria. The differences between each treatment group and the untreated group were all statistically significant.

Tobramycin has been shown to be an effective antibiotic when impregnated into cement, and other carriers. An evaluation of the elution of tobramycin from cement beads showed effective local tissue levels for up to 28 days.[9] An animal study of 10% tobramycin in calcium sulphate showed an excellent local level of antibiotic without elevated serum levels or end-organ effects.[7] Tobramycin has been shown to be effective against a broad spectrum of bacteria.[10]

Simplex P with tobramycin bone cement was introduced commercially in 2000 in Europe and in 2003 in the USA, and has a lower tobramycin concentration (1.7% *vs* 4%) than both the experimental calcium sulphate product and the hand-made PMMA beads. It performed as well in our model as the other implants but requires a second procedure to remove it and is therefore not ideal for filling contaminated bone defects.

Calcium sulphate (plaster of Paris) has been used as a bone graft substitute for over 100 years, with the first reported use by Dreesmann in 1892.[5,6] Recent reports for calcium sulphate pellets (Osteoset) used to treat cavitary bone lesions showed excellent results.[5,6] In 1998 the manufacturers of Osteoset introduced a tobramycin-impregnated product termed Osteoset-T which has been used extensively in both Europe and Canada. Our previous work showed results comparable to those for antibiotic-impregnated PMMA beads for both Osteoset impregnated with 10% tobramycin[2] and this implant mixed with demineralised bone matrix.[1] The current study was undertaken to investigate the efficacy of commercially-available Osteoset-T, which has a lower concentration of tobramycin (4% by weight).

Tobramycin-impregnated calcium sulphate pellets (Osteoset-T with 4% tobramycin) have been used effectively in cases of osteomyelitis in both the limbs and the spine.[11-14] McKee et al[13] reported their experience with this product in treating 25 infected long-bone defects and nonunions, achieving eradication of the infection in 92%. Von Stechow et al[14] used this product to treat infective spondylitis through a posterior approach, achieving excellent results in 14 of 16 cases. Gitelis and Brebach[12] reported eradication of limb osteomyelitis using hand-made Osteoset pellets containing 4% tobramycin.

This work suggests that tobramycin-impregnated calcium sulphate pellets can be effective in the treatment of contaminated fractures. In addition to being an excellent delivery medium for the antibiotic, this bone graft substitute is both bioabsorbable and osteoconductive. A second surgical procedure for their removal is not needed.

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